Rhizosphere Remediation of Recalcitrant Soil Contaminants: An Important Component of Long-term Sustained Biosystem Treatment

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Sustained Rhizosphere Remediation of Recalcitrant Contaminants in Soil: Forensic Investigations with Laboratory Confirmation

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A conservative estimate of the volume of PAH contaminated soil in the US (based on U.S. Dept. of Commerce Reports) is 1 billion ton. Biosystem Treatment relying on the integrated action of plants and microbes over extended time periods has the potential of reducing cleanup costs by >90%, and lead to pollutant degradation rather than just containment that leaves the burden of cleanup for future generations. The three components of Biosystem Treatment are plant evapotranspiration, plant-microbe rhizosphere degradation, and natural attenuation of groundwater by microbes. The least studied and therefore poorest understood of these three components is rhizosphere degradation.

Rhizosphere research in our laboratory has addressed the hypothesis: "Roots of some plant species enhance the degradation of recalcitrant, organic soil contaminants (i.e. PCBs and PAHs) by releasing cometabolites and facilitating soil aeration, both a result of fine root turn over". Published results from our laboratory supporting this hypothesis are: (1) purified natural plant compounds (i.e. flavonoids) stimulate the growth and activity of PCB and PAH degrading bacteria, (2) flavonoid compounds are present in mulberry fine roots, (3) flavonoid compounds accumulate in aging/dying mulberry roots, (4) over 50% of the fine roots turnover (die) annually. Demonstration of statistically significant reductions in the concentrations of high molecular wt, low water soluble contaminants in laboratory pot studies have failed. This is attributed to several factors: (1) pot-study artifacts (i.e. unnatural root distribution), (2) limited soil-root contact at any one time, (3) long time (several seasons) necessary for extensive soil exploration through fine root turn over. For these reasons, the only valid test of rhizosphere remediation of recalcitrant, slightly water soluble contaminants (PCBs and high molecular wt PAHs) are long term (15-20 yr) field studies. Because of the inability to gain authorization to conduct such a study we resorted to an alternative, forensic examination of naturally revegetated sites. At a revegetated former sludge basin we have shown a 50-90% reduction of PAHs (including slightly water soluble benzo(a)pyrene) in the 120 cm root zone of 12-16 yr old mulberry trees where over two hundred PAH degrading bacteria isolates have been recovered.

Currently available laboratory and forensic field data justifies initiation of carefully monitored long-term Biosystem Treatment projects. During early stages of treatment (first 5 years) the monitoring should establish that the components of the system (roots and degrading bacteria) are in place with monitoring shifting to analysis of contaminant disappearance after 5 years. It is gratifying that a Biosystem Treatment strategy has been adopted at Bofors Nobel Site in Michigan. The most pressing need to advance the Biosystem Treatment concept is improved assessment tools to monitor the biological components of the integrated system. To that end, our current research is focused on development of improved field assessment tools. Our approach is: identify working rhizosphere systems at existing revegetated sites, study the components of these working systems in the laboratory, develop assessment methods in the laboratory, and return to the field to validate, and use the newly developed methods.

Rhizosphere Remediation of Recalcitrant Soil Contaminants:
An Important Component of Long-term Sustained Biosystem Treatment

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A conservative estimate of the volume of PAH contaminated soil in the US (based on US Dept; of Commerce Reports) is 1 billion tons with some associated with former manufactured gas plant sites dating back 150 years. Biosystem Treatment relying on the integrated action of plants and microbes over extended time periods has the potential of reducing cleanup costs by > 90%. Furthermore, the end result would be pollutant degradation rather than just containment, characteristic of capped cells where unfavorable water and oxygen conditions prevent biological degradation, thus preserving the waste and leaving the burden of cleanup for future generations. Three important components of Biosystem Treatment are plant evapotranspiration, plant/microbe rhizosphere degradation, and natural attenuation of groundwater by microbes. The least studied and therefore poorest understood of these components is rhiosphere degradation.

Underpinning our research approach to phytoremediation is the realization that although plants in natural ecosystems produce polyaromatic compounds (flavaniods, coumarins, etc.) in their leaves, stems and roots, these compounds have not accumulated in the soil to concentrations reflective of their annual production over thousands of years (Figure 1). There is only a limited understanding of the production and recycling of carbon associated with naturally occurring polyaromatic compounds in terrestrial ecosystems (i.e. tannins in oak forests, Figure 1). However, it is apparent that since they have not accumulated to astronomic amounts over the last millennium, mechanisms do exist within nature to degrade and recycle carbon present in thousands of naturally occurring polyaromatic compounds many of whose structures resemble those of recalcitrant pollutants such as PCBs, and PAHs (Figure 2). The obvious question is, "Will the biological mechanisms in nature that degrade natural polyaromatic compounds also degrade recalcitrant organic pollutants (i.e. PCBs and PAHs)?" If so, what soil ecosystems associated with what plants are most active against pollutants? How should we introduce and manage these natural, multi-organismic systems to optimize their degradative properties towards recalcitrant pollutants? All of these questions deserve attention and should be resolved in order to develop dependable sustained phytoremediation technology. First however, there is a need for a better understanding of rhizosphere degradation properties and its relationship to xenobiotic compounds. To that end, our research has addressed the hypothesis: "Roots of some plant species enhance the degradation of recalcitrant, organic soil contaminants (i.e. PCBs and PAHs) by releasing cometabolites and facilitating soil aeration, both a result of fine root turn over".

The emphasis of our research over the past 15 years has been placed on understanding the mechanism of rhizosphere degradation of PCBs and PAHs with secondary attention given to the disappearance of these recalcitrant contaminants from contaminated soil. The rational for placing emphasis on mechanistic studies was that the results collected not only served to test the hypothesis but also provided a level of understanding that is necessary to improve the performance of phytoremediation and develop monitoring tools to facilitate field implementation, the importance of which is described later in this summary (Figure 3). Published results from our laboratory supporting the hypothesis and providing a mechanistic understanding of rhizosphere degedation are: (1) purified natural plant compounds (i.e. flavaniods) stimulate the growth and activity of PCB degrading bacteria (Donnelly, etal. 1994); (2) Plant roots release phenolic compounds that support the growth of PCB degrading bacteria, but all plant species are not effective (Fletcher and Hegde, 1995; Fletcher etal. 1995, Hegde and Fletcher, 1996); (3) Flavanoid compounds that support the growth of PAH- degrading bacteria accumulate in aging/dying fine roots of mulberry (Leigh, etal. 1998); (4) field studies have shown that fine mulberry roots grow in contact with PAH-contaminated sludge at 1 meter depths (Olson and Fletcher 1999). The combined interpretation of these data is that the roots of some plant species are capable of growing to immobile soil contaminants (PCBs and high mol. wt. PAHs) and deliver cometabolies (i.e. flavaniods) upon fine root death. These natural cometabolites foster the growth and activity of degredative microbes. The dead/decayed roots also create soil cavities that facilitate soil aeration. Thus, in order for roots to foster the degradation of immobile soil contaminants

(PCBs and PAHs) it is not necessary for the water insoluble contaminants to move to the root, because fine roots (<0.5mm in diameter) grow to the contaminants, and upon root death serve as soil injectors of bacterial cometabolites and facilitators of soil aeration (Figure 4). Based on this mechanistic understanding, the performance of rhizosphere remediation can be improved by increasing both root synthesis of cometabolites and the rate of fine root turnover.

Efforts on our part to demonstrate statistically significant reductions in the concentrations of high molecular wt, low water soluble contaminants in laboratory pot studies have failed. This is attributed to several factors: (1) pot-study artifacts (i.e. unnatural root distribution), (2) limited soil-root contact at any one time (<5%), and (3) long time (several seasons) necessary for extensive soil exploration through fine root tur over (growth followed by death). For these reasons, it is our contention that long-term (15-20 year) field studies are the only valid test of rhizosphere remediation of recalcitrant, slightly water-soluble contaminants (PCBs and high molecular wt PAHs). Because of the inability to gain authorization to conduct such a study we resorted to an alternative, forensic examination of naturally revegetated sites. At a revegetated former sludge basin we have shown a 50-90% reduction of PAHs (including slightly water soluble benzo(a)pyrene) in the 120 cm deep root zone of 12-16 yr old mulberry trees where over two hundred PAH degrading bacteria isolates have been recovered (Olson and Fletcher, 1999; Olson et.al., 2000.)

Based on current data available from laboratory mechanistic studies and forensic field data that have been collected on recalcitrant soil contaminants, we believe carefully monitored long-term Biosystem Treatment projects (15-20 years) should be initiated. Because of the long time required for roots to have a statistical influence on the degradation of immobile soil contaminants for reasons explained earlier, we propose that during early stages of treatment (first 5 years) the monitoring should establish that the components of the system (roots and degrading microorganisms) are in place with monitoring shifting to analysis of contaminant disappearance after 5 year (Figure 3). Monitoring the existence and operation of the degratative system instead of the product of the system (compound disappearance) is a more sensitive way to establishing that slow but sustained rhizosphere remediation is working. We are in the process of developing chemical and molecular methods to monitor the existence and function of rhizosphere degradation in the field. The development of these methods is capitalizing on basic research that was conducted in our laboratory to understand mechanistic features of the plant rhizosphere.

It is gratifying that ideas and phytoremediation data gained at the University of Oklahoma were instrumental in designing and promoting the Biosystem Treatment strategy that has been adopted at Bofors Nobel Superfund site in Michigan. Implementation of the Biosystem Treatment at Bofors will be an example of capitalizing on the combined action of plant evapotranspiration, plant-microbe rhizosphere degradation, and natural attenuation by groundwater microbes for the long-term sustained treatment of contaminants across space and time, typical of natural ecosystems.

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